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RESEARCH PAPER

Production and properties of low-glycemic index cookies from retrograded water yam starch

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Abstract

A primary challenge for functional foods, such as low-glycemic index (GI) products, is poor consumer acceptance which limits their market success. This study utilized a sensory-driven strategy, employing a Box–Behnken response surface methodology (RSM) with overall sensory score as the primary optimization criterion, to develop cookies substituting wheat flour with retrograded water yam starch (RtS). The optimized formulation (achieving a maximal predicted sensory score of 8.221) was obtained at 39.24% RtS substitution, 157.13 °C baking temperature, and 29.54 min baking time. Comparative sensory analysis revealed that the RtS cookies significantly outperformed commercially available healthy cookies in overall acceptability. Subsequent *in vivo* validation confirmed the product's functional benefit, revealing an *in vivo* GI (iGI) of 44.37, classifying it as a low-GI product (<55). These findings demonstrate the potential of RtS cookies as functional bakery items that are not only sensorially appealing but also suitable for individuals requiring controlled postprandial glycemia, including those with obesity or type 2 diabetes.

Keywords

Consumer acceptance, functional food, optimization, resistant starch, sensory analysis

Introduction

Recent years have witnessed a growing interest in managing diabetes through the modulation of postprandial glycemic response influenced by dietary carbohydrates. The glycemic index (GI) has emerged as a key metric for classifying foods based on their potential to elevate blood glucose levels. GI is a numerical ranking system that quantifies the rate at which glucose is released into the bloodstream following the consumption of carbohydrate-rich foods. Foods with a low GI (<55) are particularly beneficial for individuals with obesity, diabetes, and metabolic syndrome, aiding in the management of these health conditions (Ludwig et al. 2002). Zhao et al. (2023) reported an *in vivo* GI of 44.64 for cookies formulated with retrograded potato starch, oat bran, and inulin. Consumption of these low-GI potato cookies significantly ameliorated diabetic symptoms in a rat model of type 2 diabetes induced by a high-fat, high-sugar diet. A separate study (Aparicio-Saguilán et al. 2007) modified a traditional cookie recipe by partially substituting wheat flour with starch derived from green bananas. Study of Aparicio-Saguilán et al. (2007) found that substitution levels of 65–90% yielded cookies with acceptable sensory qualities. Furthermore, the *in vitro* GI of the optimized formulation (60.53) was significantly lower than that of the control cookies (77.62) and white bread (94). This study highlighted the potential of resistant starch (RS) to reduce the GI of cereal-based products. Corroborating these findings, other studies have demonstrated that incorporating RS-rich starches from various sources, including modified wheat starch modified white sorghum starch, and legume

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starches (Delamare et al. 2020), effectively lowered the *in vivo* GI of cookies. Report of Afandi et al (2021) further confirmed the crucial role of RS, derived from diverse sources such as green bananas, kidney beans, and sorghum, in influencing both *in vivo* and *in vitro* GI values of starchy food products (Afandi et al. 2021).

Furthermore, recent research has reinforced the recommendation of low-GI, fiber-rich foods for individuals with diabetes to promote glycemic control. This suggests that low-GI foods not only improve glucose metabolism but also ameliorate insulin resistance in diabetic patients (Del Prato et al. 1994). Järvi et al. (1999) further demonstrated that the enhanced glucose metabolism and improved lipid profiles associated with low-GI (<55) food consumption contribute to the effective management of both diabetes and cardiovascular diseases.

Water yam (*Dioscorea alata* L.), with its high protein and fiber content, rich potassium levels, and low GI ranging from 35.56 to 41.31, presents a favorable dietary option for individuals with diabetes (Oko and Famurewa 2015). Liu et al. (2019) investigated the impact of substituting wheat flour with water yam flour on the *in vitro* GI of bread. These findings collectively suggest that retrograded water yam starch can serve as a partial wheat flour substitute to effectively reduce the GI of cereal-based products.

While previous studies have noted the low-GI potential of water yam starch (Trinh et al. 2025) the primary challenge of poor sensory acceptance in low-GI bakery products remains unaddressed. Therefore, this study aimed to develop a novel, sensory-driven formulation strategy. We leveraged RSM, using consumer acceptability as the primary optimization driver, to create a functional cookie from retrograded water yam starch. The optimized product was then validated for its sensory superiority against commercial competitors and its low-glycemic classification confirmed via *in vivo* assessment.

Materials and methods

Preparation of retrograded starch (RtS)

Water yam tubers (2–3 kg/tuber) were procured from a local market. Starch extraction was performed based on a previously established method (Trinh et al. 2012).

Retrograded starch (RtS) was prepared as follows: a starch suspension (12 g starch in 400 mL water in a 1000-mL Erlenmeyer flask) was gelatinized by heating in a boiling water bath (95 °C, 30 minutes) with continuous stirring. The resulting starch gel was then autoclaved (121 °C/30 minutes) to ensure complete gelatinization. Subsequently, the gel was cooled to room temperature, spread thinly (3–5 cm) onto a stainless-steel tray (30×25 cm), covered with plastic film, and subjected to two alternating temperature cycles (4 °C/18 hours – 30 °C/6 hours). After 48 hours, the sample was thinly spread and dried in a convection oven at 50 °C until a moisture content of < 12% was achieved. Finally, the dried RtS was finely ground, sieved (120 mesh), and stored in airtight packaging under dry conditions until further use (Trinh et al. 2025).

Other ingredients

Other ingredients used in this study included: Bakers' Choice number 8 wheat flour (Interflour Vietnam); unsalted butter and whole milk (TH True Milk, Vietnam); granulated sugar (Bien Hoa Sugar Joint Stock Company, Vietnam); chicken eggs (Ba Huan Co., Ltd, Vietnam); and baking powder (AB Mauri, Vietnam).

Cookies preparation

Cookie formulations were prepared with a constant total weight of 30 g for RtS and wheat flour No. 8. The remaining ingredients (in grams) consisted of butter (15), sugar (9), fresh milk (7.5), egg (7.5), maize starch (3), baking powder (0.65), salt (0.4), xanthan gum (0.2), and vanilla extract (1). The control cookies contained 30 g of wheat flour No. 8. RtS replaced wheat flour at levels ranging from 5% to 60% according to a predefined experimental design (Table 1). Butter was brought to room temperature for 10 minutes and lightly mixed (1 minute). The remaining ingredients (excluding RtS and wheat flour) were then gradually incorporated with continuous mixing to form a smooth batter. Subsequently, RtS and wheat flour were added gradually to the batter. The complete mixture was thoroughly mixed using a Kitchenaid stand mixer (model 5KPM5) for 10 minutes to ensure homogeneity. The dough was then rested (10 °C, 20 minutes), rolled to a uniform thickness of 1.0 cm, cut into circular pieces with a diameter of 4.0 cm, and placed on parchment paper. Baking was performed in a Teka 30 electric oven with both top and bottom heating elements. The baked cookies were cooled to room temperature for 30 minutes and stored in sealed plastic bags until further analysis.

Table 1. Box-Benhken experimental design.

Sample (Run)	Factors (coded)			Factors (actual)		
	A	B	C	A	B	C
#1	-1	-1	0	5	120	27.5
#2	1	-1	0	60	120	27.5
#3	-1	1	0	5	200	27.5
#4	1	1	0	60	200	27.5
#5	-1	0	-1	5	160	15
#6	1	0	-1	60	160	15
#7	-1	0	1	5	160	40
#8	1	0	1	60	160	40
#9	0	-1	-1	32.5	120	15
#10	0	1	-1	32.5	200	15
#11	0	-1	1	32.5	120	40
#12	0	1	1	32.5	200	40
#13	0	0	0	32.5	160	27.5
#14	0	0	0	32.5	160	27.5
#15	0	0	0	32.5	160	27.5

Note: (A) RtS replacement, %, instead of b□t mì; (B) oven temperature, °C; (C) oven time, min.

Cookie thickness, diameter, and spread ratio

Cookie thickness, diameter, and spread ratio were determined as follows: a caliper was used to measure the thickness (T, mm) and diameter (D, mm) of six replicate cookies. Spread ratio (SD) was calculated using the following formula 1 (Handa et al. 2012):

$$SD = D/T \quad (1)$$

Texture profile of cookies

The texture profile was analyzed using a CT3 Texture Analyzer (Ametek Brookfield, USA) equipped with a 5 kg load cell and a three-point bend fixture (TA-TPB) with a span distance of 30 mm. Prior to testing, the instrument was calibrated for force and height using standard weights and gauge blocks provided by the manufacturer. The test settings were: mode – compression; pre-test speed – 2.0 mm/s; test speed – 1.0 mm/s; post-test speed – 10.0 mm/s; trigger load – 1.0 g; and target deformation – 80%. Textural properties assessed included hardness (g, defined as the peak force required for complete sample deformation).

Color space of cookies

The color of the cookies was evaluated using a Konica Minolta CR-400 handheld colorimeter (Japan) configured with Illuminant D65 and a 2° standard observer. Before the analysis, the instrument was calibrated using a standard white tile (Y = 93.5, x = 0.3155, y = 0.3319). Measurements were performed in triplicate at different locations on the cookie surface to determine CIE Lab color space values (L*, a*, and b*). L* represents lightness, ranging from 100 (absolute white) to 0 (black); a* represents the red-green axis, ranging from -a* (green) to +a* (red); and b* represents the yellow-blue axis, ranging from -b* (blue) to +b* (yellow). Color differences between samples were quantified using the ΔE value, calculated according to the following formula 2 (Francis and Clydesdale 1975).

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (2)$$

Where: $\Delta L = L^*_{sample} - L^*_{control\ sample}$; $\Delta a = a^*_{sample} - a^*_{control\ sample}$; $\Delta b = b^*_{sample} - b^*_{control\ sample}$.

Sensory evaluation of cookies

A hedonic rating test was employed to assess consumer preference for the texture, color, aroma, taste, and overall acceptability of cookies. Eighty untrained panelists (males and females, aged 18–25 years) were recruited from Ho Chi Minh City University of Technology and Education. The panelists were selected based on their regular consumption of cookies and lack of allergies to wheat, gluten, milk, or eggs. Prior to the evaluation, the study protocol was explained, and written informed consent was obtained from all participants. The sensory evaluation procedure was conducted in accordance with the ethical guidelines of the university.

The evaluation was conducted in individual sensory booths under white light at controlled room temperature (25±1 °C). The following cookies were included in the sensory evaluation: RtS cookies (optimized formula), Gullon cookies (Gullon zero sem acúcares adicionados digestive aveia, Spain), Oattie black rice cookies (Oattie black rice oat cookie, Malaysia), and Oattie chia seed cookies (Oattie chia seeds oat cookie, Malaysia). Cookie samples were served on white plates coded with three-digit random numbers. To minimize order effects, the presentation order of the samples was randomized. Panelists were provided with room-temperature water to rinse their palates between samples. They were asked to rate their liking using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely).

Response surface methodology (RSM)

A response surface methodology, employing a Box-Behnken design (Ferreira et al. 2007), was utilized to evaluate the influence and interaction of three independent variables (Table 1): (A) RtS substitution level (percentage of wheat flour replaced), (B) baking temperature (°C), and (C) baking time (minutes) on the sensory quality and physical properties (diameter [mm], thickness [mm], spread ratio, and hardness [g]) of the cookies. Given the importance of sensory attributes in determining consumer acceptance, sensory score (Y1) was selected as the major response variable (i.e., the primary target for optimization in this study). Other response variables included cookie diameter (Y2), thickness (Y3), spread ratio (Y4), hardness (Y5), and color parameters for both the top (L* - Y6, a* - Y7, b* - Y8) and bottom (L* - Y9, a* - Y10, b* - Y11) surfaces of the cookies. These additional responses (Y2 to Y11) were treated as technical parameters and modeled concurrently with the major response variable. This approach enables the prediction of sensory quality and other technical attributes based on any single measured parameter, providing valuable insights for practical cookie production. A Design Expert software version 13.0.5.0 was used to build the Box-Behnken design mentioned above.

In vitro starch hydrolysis index (SHI)

The *in vitro* starch hydrolysis index (SHI) of the cookies was determined based on a modified version of a published method (Arp et al. 2020). An enzyme solution was prepared by adding 0.1 mL of alpha-amylase to 100 mL of 0.5 M sodium phosphate buffer (pH 6.9) containing 0.05 g of NaN_3 (to inhibit microbial growth during the reaction). Prior to use, the enzyme solution (10 U/mL) was equilibrated at 37 °C for 15 minutes in a water bath. Starch

samples (0.5000 g) were suspended in 25 mL of sodium phosphate buffer and equilibrated at 37 °C for 15 minutes in a water bath with magnetic stirring. Hydrolysis was initiated by adding 5 mL of the enzyme solution to the starch suspension, and the reaction was allowed to proceed for 480 minutes. Aliquots (1.0 mL) of the reaction mixture were withdrawn at various time intervals and added to Eppendorf tubes containing 1 mL of DNS reagent (prepared by dissolving 1 g of DNS, 30 g of sodium potassium tartrate, and 1.6 g of NaOH in distilled water to a final volume of 100 mL). The mixture was then heated in a boiling water bath for 5 minutes, cooled to room temperature, and the absorbance was measured at 530 nm for reducing sugar estimation. The degree of hydrolysis (DH) was determined using calculated by using a glucose standard curve. The actual DH curve (DH vs. reaction time) was then used to derive a non-linear regression equation for DH using Minitab statistical software (version 21, USA) based on the Isabel Goñi equation (3) (Goñi et al. 1997):

$$C = C_{\infty}\left(1 - e^{-kt}\right) \quad (3)$$

Where C represents the mg of maltose released per g of starch at a specific time point, C_{∞} represents the maximum value of C (the value of C at t_{∞}), and k is the rate constant.

To estimate the SHI, the area under the curve (AUC) of the DH curve (derived from the regression equation) was calculated using Origin 8.5.1 software. The SHI was then determined as the percentage ratio between the AUC of the cookie sample and that of white bread (reference sample, GI = 100, Otto, Vietnam).

In vivo glycemic index (iGI)

Six-week-old male albino mice (*Mus musculus*) weighing 15–18 g were obtained from the Ho Chi Minh City Pasteur Institute, Vietnam. Prior to experimentation, the mice were acclimated to a standard diet (ND) until they reached a weight of 30±1 g. Throughout the study, animals were housed under standard laboratory conditions (30±1 °C, 60–70% humidity, 12-hour light/dark cycle) with ad libitum access to food and water to ensure optimal growth and development (Reeves 1997). Drinking water was supplemented daily with a few drops of vitamin solution (Hagrbuttentrunk, Beaphar, Netherlands). The sample size (n = 7) was calculated following a previous study (Charan and Kantharia 2013).

The *in vivo* glycemic index (iGI) was assessed using male mice fasted for 12 hours (overnight with free access to water). Mice were then administered 0.5 mL of a 7.5% (w/v) suspension of either white bread (reference sample) or cookies via oral gavage. Blood samples (one drop) were collected from the tail vein at 0 minutes (pre-administration) and 15, 30, 60, 90, 120, and 180 minutes post-administration. Blood glucose levels were measured using a BeneCheck glucose meter (General Life Biotechnology Co. Ltd, Taiwan) (Shin et al. 2007; Trinh et al. 2025). The area under the curve (AUC) for blood glucose versus time was calculated using Origin 8.5.1 software (OriginLab Corporation, Northampton, USA). The iGI was then determined as the percentage ratio between the AUC of the cookie sample and that of white bread (reference sample, GI = 100, Otto, Vietnam). The *in vivo* procedures were approved by the Ethics Committee of Dinh Tien Hoang Medical Research Institute (Certificate No. IRB-A-2301) and were conducted in accordance with established bioethical standards.

Statistical analysis

All measurements were at least triplicate, and the results (mean ± standard deviation) were shown after processing. The analysis of ANOVA variance method and the differences in mean values were determined by Tukey's test ($p < 0.05$) using Minitab Statistical Software (Version 18, Minitab, LLC).

Results and discussions

The experimental results of the responses based on the Box-Behnken model were presented in Table 2 and the regression equations of the responses were described in Table 3. In addition, we analyzed the correlations between the responses and presented them as contour (2D) plots (Fig. 2) and surface (3D) plots (Fig. 1). Analysis of the regression equations (Table 3) revealed that all models exhibited high coefficients of determination ($R^2 > 0.99$) and insignificant lack of fit ($p > 0.05$). These results indicate that the models adequately describe experimental data and are statistically significant.

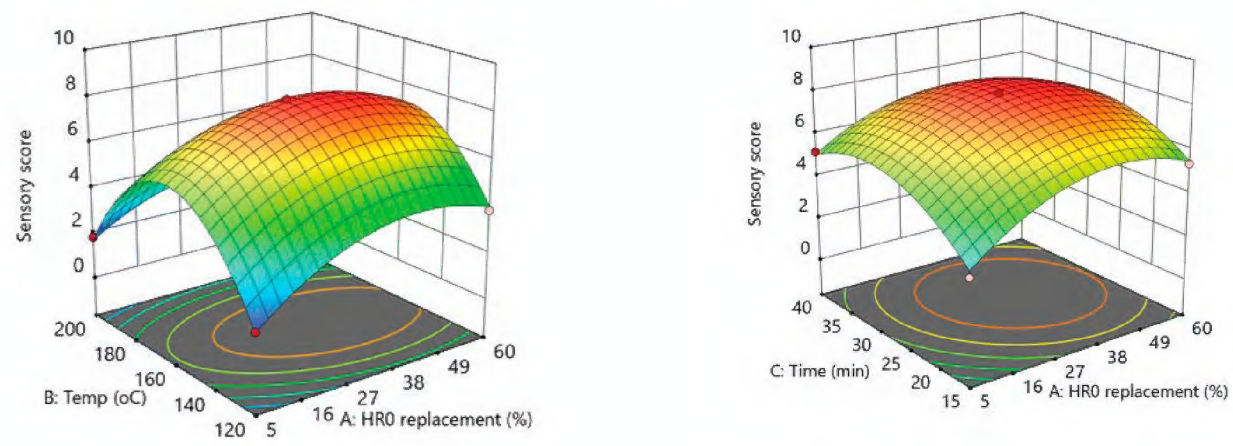

Figure 1. 3D surface plot of Y1 response. Note: (A) RtS replacement, %, instead of wheat flour; (B) oven temperature, °C); (C) oven time, min; (Y1) sensory score.

Table 2. Responses of Box-Benhken design.

Sample (Run)	Responses										
	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11
#1	1.65±0.64^{e}	44.98±0.04^{b}	9.26±0.06^{h}	4.87±0.03^{e}	213.4±40.3^{ij}	74.56±0.43^{c}	2.19±0.11^{f}	33.08±0.13^{de}	77.70±0.20^{a}	-1.02±0.02^{m}	33.26±0.07^{e}
#2	3.86±0.87^{c}	44.43±0.02^{d}	8.15±0.04^{l}	5.45±0.02^{a}	139.9±26.0^{jk}	71.51±0.46^{e}	0.60±0.06^{g}	33.11±0.06^{d}	74.13±0.64^{c}	0.40±0.14^{k}	33.14±0.09^{e}
#3	1.84±0.77^{e}	43.32±0.02^{j}	12.29±0.02^{b}	3.52±0.01^{l}	3000.8±38.9^{b}	51.95±0.62^{j}	8.73±0.28^{b}	27.82±0.37^{h}	50.51±0.23^{i}	10.49±0.27^{b}	30.93±0.21^{g}
#4	2.26±1.06d^{e}	43.03±0.01^{k}	8.61±0.03^{j}	5.00±0.02^{c}	1682.3±40.9^{d}	48.24±0.33^{k}	9.49±0.41^{b}	27.78±0.14^{h}	47.44±0.34^{j}	10.14±0.30^{b}	31.93±0.38^{f}
#5	3.03±0.81^{cd}	45.04±0.02^{a}	11.57±0.03^{c}	3.89±0.01^{k}	655.2±38.6^{g}	77.43±0.65^{a}	3.51±0.38^{e}	35.14±0.19^{bc}	76.35±0.37^{b}	2.49±0.26^{h}	34.47±0.33^{c}
#6	5.38±1.89^{b}	43.94±0.02^{f}	9.62±0.02^{g}	4.57±0.01^{g}	259.1±39.9^{i}	74.51±0.64^{c}	3.24±0.22^{e}	35.75±0.62^{b}	73.54±0.32^{c}	1.96±0.10^{i}	35.22±0.23^{b}
#7	5.18±1.03^{b}	43.51±0.01^{h}	12.45±0.05^{a}	3.49±0.02^{l}	2580.0±43.1^{c}	64.15±0.32^{h}	9.07±0.24^{b}	29.55±0.11^{g}	62.13±0.19^{g}	8.60±0.20^{c}	29.14±0.06^{i}
#8	6.18±1.51^{ab}	43.41±0.01^{i}	9.06±0.03^{i}	4.79±0.02^{f}	1223.1±34.0^{e}	61.27±0.41^{i}	7.74±0.37^{c}	29.40±0.26^{g}	59.24±0.20^{h}	8.16±0.15^{d}	29.20±0.11^{i}
#9	1.86±0.65^{e}	44.33±0.01^{e}	8.46±0.04^{k}	5.24±0.02^{b}	111.0±23.9^{k}	76.03±0.24^{b}	2.73±0.12^{ef}	34.61±0.40^{c}	77.48±0.27^{a}	-0.43±0.07^{l}	34.04±0.06^{d}
#10	2.36±1.19^{de}	44.70±0.01^{c}	10.58±0.03^{d}	4.22±0.01^{h}	909.5±20.7^{f}	67.81±0.19^{g}	4.82±0.42^{d}	36.76±0.31^{a}	66.45±0.38^{f}	4.66±0.13^{g}	38.89±0.07^{a}
#11	3.93±0.73^{c}	45.05±0.03^{a}	9.14±0.03^{i}	4.93±0.02^{d}	374.7±32.6^{h}	73.25±0.52^{d}	1.05±0.45^{g}	34.91±0.22^{c}	75.78±0.33^{b}	0.81±0.08^{j}	34.38±0.24^{cd}
#12	1.53±0.64^{e}	42.00±0.01^{l}	10.47±0.03^{e}	4.01±0.01^{j}	3168.5±41.4^{a}	35.50±0.35^{l}	14.33±0.65^{a}	24.38±0.38^{i}	34.53±0.12^{k}	13.87±0.13^{a}	26.86±0.11^{j}
#13	8.04±0.93^{a}	43.95±0.03^{f}	10.42±0.09^{ef}	4.22±0.04^{h}	1195.0±47.6^{e}	69.39±0.05^{f}	5.40±0.22^{d}	32.44±0.16^{ef}	67.94±0.32^{d}	5.42±0.05^{e}	30.48±0.16^{h}
#14	8.22±0.82^{a}	43.95±0.03^{f}	10.36±0.03^{f}	4.24±0.02^{h}	1167.5±49.9^{e}	68.83±0.55^{f}	4.90±0.81^{d}	32.04±0.23^{f}	67.54±0.48^{de}	5.20±0.23^{ef}	30.37±0.22^{h}
#15	7.97±0.83^{a}	43.88±0.02^{g}	10.58±0.03^{d}	4.15±0.01^{i}	1258.0±33.8^{e}	68.55±0.16^{fg}	4.70±0.16^{d}	31.83±0.14^{f}	67.16±0.20^{ef}	4.98±0.16^{fg}	30.12±0.08^{h}

The values are presented as Mean ± standard deviation. Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

Note: (A) RtS replacement, %, instead of wheat flour; (B) oven temperature, °C); (C) oven time, min; (Y1) sensory score; (Y2) diameter, mm; (Y3) thickness, mm; (Y4) spread rati; (Y5) hardness, g; (Y6) L* top; (Y7) a* top; (Y8) b* top; (Y9) L* bottom; (Y10) a* bottom; (Y11) b* bottom.

Table 3. Regression equations of responses.

Response	Regression equation (actual values)	R^2	Lack of fit (>0.05)
Y1	= -76.99357 + 0.2547A + 0.8624B + 0.8543C – 0.000407AB – 0.00145BC - 0.002084A^2 – 0.002561B^2 – 0.009973C^2	0.9942	0.0994
Y2	= 42.39742 – 0.037366A + 0.0213345B + 0.186977C + 0.000727AC - 0.00171BC	0.9961	0.1251
Y3	= -11.74530 + 0.070336A + 0.240336B + 0.052163C – 0.000584AB – 0.001047AC – 0.000395BC– 0.00059B^2	0.9971	0.4890
Y4	= 14.47257 – 0.0304699A – 0.111727B – 0.0073488C + 0.000205AB + 0.000444AC + 0.000288B^2	0.9953	0.2890
Y5	= -1662.42216 + 46.29836A + 5.13227B – 57.16132C – 0.282970AB – 0.698771AC + 0.997665BC	0.9927	0.0607
Y6	= -36.43919 + 0.016011A + 1.47901B + 1.39229C – 0.014761BC - 0.004235B^2	0.9951	0.0556
Y7	= 1.4961 – 0.036287A + 1.01575B + 1.55462C – 0.01511BC - 0.002924B^2	0.9982.	0.1123
Y8	= 16.14770 – 0.084870B – 0.933069C + 0.005595BC	0.9951	0.3787
Y9	= -11.60691 + 0.103861B – 0.381767C + 0.003985BC	0.9919	0.0638
Y10	= 12.94421 + 0.261002B + 0.339697C – 0.006340BC – 0.001233A^2 – 0.000454B^2 + 0.008227C^2	0.9951	0.3186
Y11	= 48.7597 – 0.218383B + 0.289056C – 0.006685BC + 0.001180B^2 + 0.010117C^2	0.9949	0.1365

Note: (A) RtS replacement, %, instead of wheat flour; (B) oven temperature, °C); (C) oven time, min; (Y1) sensory score; (Y2) diameter, mm; (Y3) thickness, mm; (Y4) spread rati; (Y5) hardness, g; (Y6) L* top; (Y7) a* top; (Y8) b* top; (Y9) L* bottom; (Y10) a* bottom; (Y11) b* bottom.

Sensory score

As depicted in Fig. 1, all three factors (A, B, and C) significantly influenced the response variable Y1 (sensory score). The maximum sensory score ($Y1_{max}$ = 8.221) was achieved at an RtS substitution level of 39.243%, a baking temperature of 157.13 °C, and a baking time of 29.54 minutes. Fig. 1 further illustrates that increasing the RtS substitution level (factor A) from 5% to 39.24% resulted in a gradual increase in sensory score. However, further increases in the substitution level up to 60% led to a decline in sensory score. This observation is consistent with previous findings indicating that, compared to conventional dietary fibers, resistant starch exerts a more favorable impact on cookie properties such as appearance, texture, and mouthfeel (Sajilata et al. 2006). Nevertheless, excessive RtS content can impart a gritty texture, stickiness, and difficulty in swallowing. Moreover, it can result in increased hardness, decreased crispness, a crumbly texture, and unacceptable deviations in the physical properties of the cookies.

Increasing the baking temperature (factor B) from 120 °C to 157.132 °C resulted in a progressive enhancement of the sensory score (Fig. 1). This observation aligns with previous studies reporting that color and aroma development in baked goods are primarily driven by Maillard reactions and caramelization. The concentration and diversity of Maillard reaction products increase with temperature up to approximately 160 °C, leading to desirable golden-brown coloration and enhanced flavor in cookies baked within this range. However, further increases in baking temperature resulted in a decline in sensory scores. This can be attributed to excessive browning, burnt flavors, bitterness, and increased hardness, rendering the cookies less appealing to the panelists. Baking time (factor C) also significantly impacted cookie quality. Extending the baking time from 14 to 29.54 minutes led to a gradual improvement in sensory scores, likely due to optimal moisture removal and the development of characteristic porous structures (Panghal et al. 2018). However, further increases in baking time up to 40 minutes caused a decrease in sensory scores. Prolonged baking can lead to the formation of undesirable Maillard reaction products that impart off-flavors and potentially pose health risks.

To validate the predictive accuracy of the model, a cookie sample was prepared using the optimized conditions:

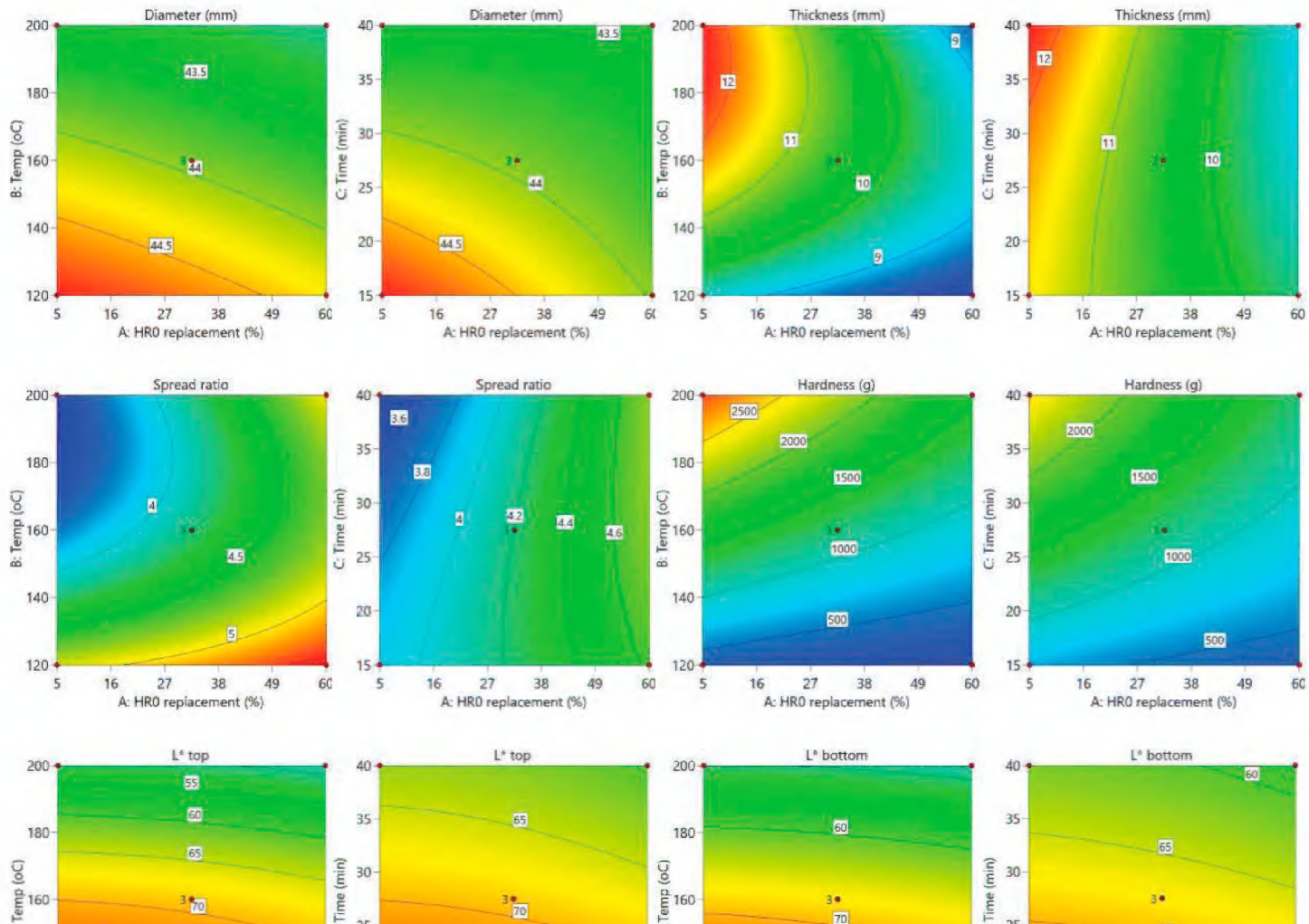

Figure 2. Contour (2D) plot of reponses (from Y2 to Y11). Note: (A) RtS replacement, %, instead of wheat flour; (B) oven temperature, °C); (C) oven time, min; (Y1) sensory score; (Y2) diameter, mm; (Y3) thickness, mm; (Y4) spread rati; (Y5) hardness, g; (Y6) L* top; (Y7) a* top; (Y8) b* top; (Y9) L* bottom; (Y10) a* bottom; (Y11) b* bottom.

RtS substitution level of 38.8%, baking temperature of 157 °C, and baking time of 29.5 minutes. Sensory evaluation of this sample yielded a mean sensory score of 8.02±0.89. A two-sample t-test revealed no significant difference ($p > 0.05$) between the predicted and actual sensory scores, confirming the validity and predictive capability of the developed model.

Diameter of cookies

Fig. 2 illustrates an inverse relationship between cookie diameter (Y2) and all three factors (A, B, and C). However, the observed variations in diameter were relatively small and unlikely to substantially impact overall cookie quality. The decrease in diameter with increasing baking temperature (120 °C to 200 °C) can be attributed to moisture loss. At temperatures exceeding 190 °C, rapid starch gelatinization and protein coagulation increase dough viscosity, hindering cookie spread. Higher temperatures also promote premature gas release, resulting in denser, harder cookies with reduced diameters. Similarly, extending baking time from 15 to 40 minutes led to a gradual reduction in diameter as the outer layers solidify, causing the internal structure to contract. Furthermore, air bubbles incorporated during mixing escape during baking, and prolonged baking leads to further air and moisture loss, contributing

to the observed decrease in diameter. These findings are consistent with previous work that demonstrated the significant influence of baking time and temperature on heat transfer and the quality of baked goods. The complex physicochemical interactions among ingredients during baking contribute to variations in texture, aroma, and color. In addition to the effects of temperature and time, the partial substitution of wheat flour with RtS also contributed to the slight reduction in cookie diameter, likely due to decreased dough elasticity (Wilderjans et al. 2008).

Thickness

Fig. 2 demonstrates that cookie thickness is inversely proportional to RtS substitution level (factor A) and directly proportional to both baking temperature (factor B) and baking time (factor C). The observed decrease in thickness with increasing RtS incorporation can be attributed to a reduction in the gluten network within the dough, leading to a drier texture and reduced spread (Laguna et al. 2011). While increasing the baking temperature from 120 °C to 160 °C led to a gradual increase in cookie thickness, further increases up to 200 °C resulted in a decrease. This phenomenon can be explained by the rapid increase in dough viscosity at temperatures above 190 °C due to accelerated starch gelatinization and protein coagulation, which restrict cookie spread (Panghal et al. 2018). Similarly, cookie thickness exhibited a positive correlation with baking time. Prolonged baking induces significant changes in cookie composition, including alterations in starch gelatinization kinetics, moisture content, and protein coagulation. Moreover, extended heat exposure promotes greater water evaporation, contributing to the observed increase in thickness (Panghal et al. 2018).

Spread ratio

As shown in Fig. 2, spread ratio is directly proportional to RtS substitution level (factor A) and inversely proportional to both baking temperature (factor B) and baking time (factor C). Increasing the RtS substitution level reduces the gluten protein content in the dough, weakening the gluten network and decreasing dough elasticity. This results in greater dough expansion during baking and a corresponding increase in spread ratio (Pareyt and Delcour 2008). Conversely, spread ratio exhibited a decreasing trend with increasing baking temperature from 120 °C to 160 °C. This inverse relationship can be attributed to the positive correlation between baking temperature and cookie thickness, as previously discussed. Similarly, extending the baking time from 15 to 40 minutes led to a gradual reduction in spread ratio, consistent with previous findings (Panghal et al. 2018). This decrease can be explained by the increase in cookie thickness with prolonged baking, which restricts dough expansion. Cookies undergo significant expansion early in the baking process due to CO_2 release from baking powder and water evaporation. The extent of CO_2 diffusion is governed by the rate of spread and the setting time of the dough. Therefore, as baking time increases, the cookie structure solidifies, hindering CO_2 diffusion and leading to a decrease in spread ratio.

Hardness

Fig. 2 illustrates that cookie hardness is directly proportional to both baking temperature and baking time. Although a slight decreasing trend in hardness was observed with increasing RtS substitution levels, this effect was not statistically significant. The addition of retrograded water yam starch reduces the gluten content in the dough, leading to a decrease in hardness due to a crumblier texture. This finding is consistent with previous research by (Belorio et al. 2019), who reported a reduction in cookie hardness with increasing corn flour content. The observed increase in hardness with increasing baking temperature (120 °C to 200 °C) can be attributed to enhanced water migration from the cookie core to the surface, promoting a firmer texture. Consequently, hardness also increased with extended baking time.

Lightness

Fig. 2 demonstrates that both L*top and L*bottom (measures of lightness) are inversely proportional to baking temperature and baking time, while remaining largely unaffected by RtS substitution level. During baking, caramelization and Maillard reactions lead to the formation of melanoidins, resulting in a darkening of the product with increasing temperature (Manzocco et al. 2000). Furthermore, excessive water evaporation during baking can intensify Maillard reactions and caramelization, further influencing color development (Chung et al. 2014). Consequently, higher baking temperatures and longer baking times resulted in darker cookies. A slight decrease in lightness was observed on both cookie surfaces with increasing RtS substitution, indicating a subtle darkening effect.

Red color

Fig. 2 indicates that the red color components (a*top and a*bottom) of the cookies are directly proportional to both baking temperature and baking time (factor C), while remaining largely independent of RtS substitution level. This observation can be attributed to the intensification of Maillard reactions at higher baking temperatures and longer baking times, leading to increased formation of melanoidin pigments. Consequently, red pigments become more dominant compared to green pigments (Manzocco et al. 2000).

Yellow color

Fig. 2 shows that the yellow color components (b*top and b*bottom) of the cookies are directly proportional to baking temperature and time, while exhibiting minimal

dependence on RtS substitution level. Increasing the baking temperature from 120 °C to 160 °C led to a gradual increase in b* values. However, further increasing the temperature from 160 °C to 200 °C resulted in a decreasing trend. This observation aligns with previous findings by (Panghal et al. 2018), who reported that while Maillard reactions occur throughout the cookie, the transition from yellow to brown coloration is more pronounced at the surface due to higher temperatures and lower moisture content. Consequently, the cookie crust exhibited a progressive darkening of yellow pigmentation with increasing baking temperature and time.

Properties of $Y1_{max}$ cookie (RtS cookie)

Based on the experimental data and parameters of the Box-Behnken model, the optimized RtS cookie formulation was produced using the following parameters: 39.24% RtS substitution, 157.13 °C baking temperature, and 29.54 minutes baking time. The physical properties of this optimized cookie are presented in Table 4. A two-sample t-test revealed no significant difference ($p > 0.05$) between the predicted (Y_p) and actual (Y_a) values for the measured properties. This confirms the agreement between the model predictions and the experimental results.

Table 4. Properties of RtS cookies (at $Y1_{max}$).

Properties	Predicted values (Y_p)	Actual values (Y_a)
Hardness (g)	1140.807	1170.67±42.09
Diameter (mm)	43.875	43.90±0.06
Thickness (mm)	10.101	10.17±0.06
Spread ratio	4.350	4.32±0.03
L*top	68.150	68.03±0.15
a*top	4.988	4.93±0.09
b*top	31.804	31.61±0.22

Sensory evalution of RtS cookies ($Y1_{max}$) and various commercially available healthy

The results of the hedonic rating test for cookies are presented in Table 5. RtS cookies received higher ratings compared to commercially available cookies supplemented with other fiber-rich plant flours. Specifically, the RtS cookies scored highest in terms of texture, color, aroma, taste, and overall acceptability, indicating strong consumer acceptance. While the incorporation of gluten-free flours can often negatively impact the sensory qualities of baked goods and reduce consumer liking, the perceived health benefits of these products can contribute to greater acceptance.

Degree of hydrolysis (DH) và *in vitro* estimated glycemic index (e) of cookies

The results indicate that white bread exhibited the fastest hydrolysis rate, followed by the control cookie, while the RtS cookie demonstrated the slowest hydrolysis rate (Fig. 3). Under the action of digestive enzyme, starch hydrolysis in all samples increased with hydrolysis time, with a rapid increase

Table 5. Sensory evaluation of RtS-substituted cookies compared to commercial healthy cookies.

Cookies	Texture	Color	Smell	Taste	Overal acceptance
RtS	7.27±1.04^{a}	7.61±0.87^{a}	7.60±0.96^{a}	7.72±0.98^{a}	7.78±0.98^{a}
Gullon	6.00±1.07^{a}	6.38±0.90^{ab}	5.00±0.86^{b}	4.37±1.01^{b}	4.50±1.05^{b}
Oattie chia sead	6.52±1.03^{a}	4.95±1.06^{b}	6.48±1.08^{ab}	6.55±1.00^{ab}	6.17±0.96^{ab}
Oattie black rice	6.55±0.96^{a}	6.77±1.03^{ab}	6.53±0.81^{ab}	6.85±1.26^{ab}	6.65±0.97^{ab}

Values are presented as mean ± standard deviation (SD). Different letters within a column indicate significant differences ($p < 0.05$) according to a post-hoc test. The following cookies were included in the analysis: RtS cookies (optimized formula); Gullon cookies (Gullon zero sem acúcares adicionados digestive aveia, Spain); Oattie black rice cookies (Oattie black rice oat cookie, Malaysia); and Oattie chia seed cookies (Oattie chia seeds oat cookie, Malaysia).

observed within the first 20 minutes. For both white bread and the control cookie, the degree of hydrolysis (DH) plateaued after approximately 400 minutes. In contrast, the DH of the RtS cookie reached a plateau after only 60 minutes. As shown in Table 6, the starch hydrolysis index (SHI) of the RtS cookie was 13.07, significantly lower than the control cookie (52.96) and white bread (100.00). This indicates that the RtS cookie has a much lower relative rate of *in vitro* starch digestion by alpha-amylase compared to the reference products.

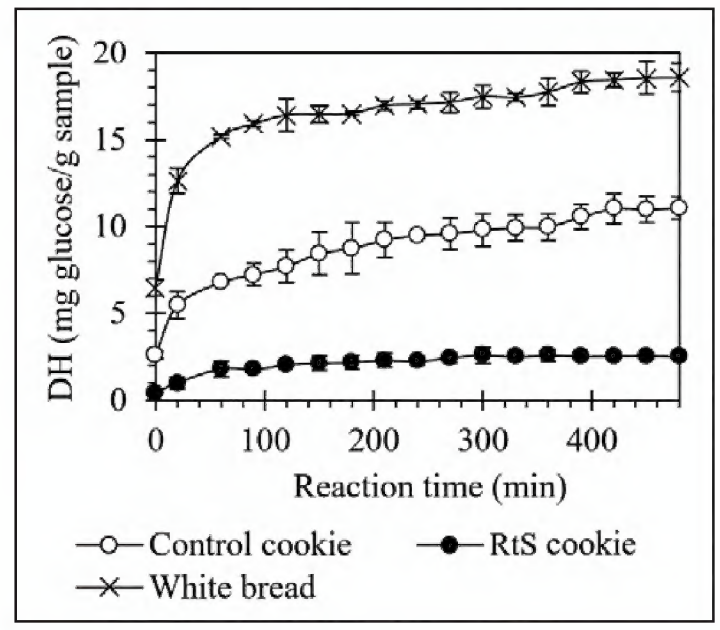

Figure 3. DH curves of cookies.

Table 6. SHI and iGi of cookies.

Sample	C_∞ (mg/g)*	k (1/min)*	SHI	iGI
White bread	18.50	0.040	100.00^{c}	100.00^{c}
Control cookie	10.45	0.016	52.96^{b}	71.68^{b}
RtS cookie	2.60	0.010	13.07^{a}	44.37^{a}

*Parameters of Isabel Goñi's equation.

In vivo glycemic index (iGI) of cookie supplemented with RtS

Fig. 4 illustrates that at the initial time point, blood glucose levels in all mouse groups were statistically similar ($p < 0.05$) and within the normal range (~86.47 mg/dL). Following the administration of sample slurries, blood glucose levels increased rapidly, reaching peak values at 30 minutes. Throughout the monitoring period, blood glucose levels consistently followed a decreasing order: white bread > control cookie > RtS cookie. After 30 minutes, blood glucose levels gradually declined in all groups, returning to baseline levels by 180 minutes. The extent of starch digestion and absorption is reflected in the blood glucose response over the 180-minute postprandial period.

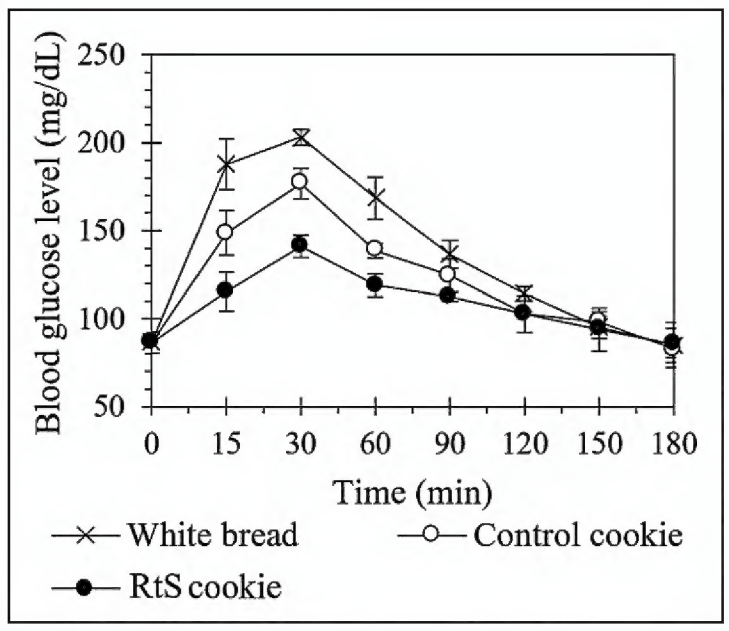

Figure 4. Blood glucose levels in mice groups fed white bread, control cookies, and RtS cookies.

The SHI and iGI values followed a similar trend across the samples, decreasing in the following order: white bread > control cookie > RtS cookie. However, the SHI (*in vitro*) consistently yielded lower values compared to the iGI (*in vivo*). This discrepancy is expected, as the simple *in vitro* hydrolysis model using only alpha-amylase (SHI) does not capture the full complexity of the *in vivo* digestive process, which involves multiple enzymes and physiological factors. While *in vitro* methods aim to simulate *in vivo* conditions, it remains challenging to precisely replicate the complexities of human or animal digestive systems. Consequently, the iGI of the RtS cookie (44.37) was higher than its SHI (13.07). This difference likely stems from physiological factors (digestive function, glucose tolerance, food consumption rate) and meal-related factors (physical form, presence of other nutrients) that influence *in vivo* digestion but are not fully accounted for in simplified *in vitro* assays (Jenkins et al. 2002). Previous research has established gastric emptying rate as a key determinant of the rate at which ingested food becomes available for digestion in the small intestine. Factors such as acidity, osmolarity, volume, and sugar concentration influence gastric emptying. However, *in vitro* methods are limited in their ability to capture the effects of variations in gastric emptying and the impact of viscosity on the absorption rate of digestion products (Brand-Miller and Holt 2004). Therefore, *in vitro* tests such as the one used here provide an estimate of relative starch digestibility, while *in vivo* tests offer a definitive assessment of the actual glycemic response. Nonetheless, *in vitro* methods remain valuable for specific applications, such as new product development and investigating factors influencing carbohydrate digestion rates. Accurate information on the GI of foods is crucial for consumers, healthcare professionals, and individuals with diabetes to make informed dietary choices.

The glycemic index (GI) is used to classify carbohydrate-rich foods into three categories: low (<55), medium (56–69), and high (>70). Based on this classification, the *in vivo* GI (iGI) of the RtS cookie (44.37, as shown in Table 6) confirms it falls within the low-GI category.

Previous research Järvi et al. (1999) demonstrated that low-glycemic index diets not only ameliorate certain metabolic consequences of insulin resistance but also directly reduce insulin resistance itself. Furthermore, the improvements in glucose metabolism and blood lipid profiles associated with low-GI food consumption have been shown to exert positive effects on the management of both diabetes and cardiovascular diseases.

Conclusion

This study employed a Box-Behnken optimization model to determine the optimal conditions for producing RtS cookies with the highest sensory scores. When compared to several commercially available diet cookies, the RtS cookies exhibited significantly better overall acceptability, suggesting promising consumer acceptance. Furthermore, the RtS cookies demonstrated the ability to reduce postprandial blood glucose levels. These findings indicate that the RtS cookies, with their low glycemic index, are suitable for individuals on weight management programs, those with obesity, and those with type 2 diabetes.

Competing interests

The authors have declared that no competing interests exist.

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